Engineering *Deinococcus radiodurans* for Actinide Bioprecipitation

Cynthia-May S. Gong^{1,2}, Jay D. Keasling², Heino Nitsche^{1,2}

¹Nuclear Sciences Division, Lawrence Berkeley National Laboratory, Building 70, Berkeley, CA 94720 ²College of Chemistry, University of California at Berkeley, ³Life Sciences Division, Lawrence Berkeley National Laboratory. HNitsche@lbl.gov. phone: (510) 486-5209 fax: (510)486-7444

Deinococcus radiodurans, a nonpathogenic prokaryote able to withstand high doses of ionizing radiation, is being engineered for bioremediation and biostabilization of heavy metals and actinides. An actinide bioprecipitation system for *D. radiodurans* capable of polyphosphate accumulation, inducible degradation and secretion, and ${\rm UO_2}^{2+}$ precipitation, is being developed.

The radioresistance of *D. radiodurans* to ionizing gamma radiation has been well studied, yet is not well understood. Several DNA repair mechanisms have been discovered, yet survivability studies are typically done under nutrient-rich conditions at a single growth stage. Although a minimal irradiation medium has been described, the effect of different variables on survivability in a nutrient-starved environment has not been systematically studied. Results indicate that there is a threshold below which the radiation dose is not sufficient to overcome the cells' passive defense mechanisms, such as radical scavenging and constitutive repair proteins. Additional radiation survivability is then from active resistance induced by cell damage, possibly related to aging. The effects of light-ion irradiation in aqueous suspension have also been studied using the 88" cyclotron at the Lawrence Berkeley National Laboratory and increasing lethality corresponding to increasing linear energy transfer (LET) values of the radiation is seen. Previous studies only examined heavy ion effects on cells supported on solid medium.

To better understand the sorption chemistry of *D. radiodurans*, the interaction of strain R1 with UO₂²⁺ has been studied. The Gram-negative R1 uranyl sorption load is an order of magnitude less than that of non-engineered Gram-positive Bacillus sphaericus under similar conditions, and more than two orders of magnitude less than a polyphosphate accumulation engineered strain of Gram-negative Pseudomonas aeruginosa. Chemical studies of the cell-uranyl binding strength and pH sorption edges support spectroscopic data indicating that a carboxyl surface group, consistent with known characteristics of *D. radiodurans*' S-layer, interacts with and binds the uranyl. A strain engineered with the putative polyphosphate accumulation genes shows promise for use in applications of uranyl bioprecipitation and its efficacy and contrast to the non-engineered strain will be discussed.